Indirect Method for Measurement of Pressure in Blood Capillaries
By M. Intaglietta, Ph.D., and B. W. Zweifach, Ph.D.

ABSTRACT
The capillary hydrostatic pressure of blood was measured indirectly. We used a microneedle to occlude exposed mesenteric capillaries in rats and frogs without trauma and then determined the motion of the trapped red blood cells that resulted from the absorption or filtration of water through the capillary wall.

The trajectory of red cells was determined from enlarged timed photomicrographs that cover both sides of the occlusion. Gain or loss of water was measured by following the trajectory of two or more red cells to avoid errors due to injury and incomplete occlusion and extrapolating the motion to the instant of occlusion.

Pressures were calculated from Starling's formula for fluid exchange. We assumed that the tissue parameters are an unknown constant in acute preparations; we used available values of filtration coefficients and colloidal osmotic pressures. The results show that 70 to 80% of the capillaries tested lost fluid, and therefore, their blood hydraulic pressure was higher than colloidal osmotic pressure. The pressures calculated in this way were similar to those measured by techniques requiring puncture of the capillary wall. Capillary arteriovenous pressure differences were approximately 4 mm Hg.

ADDITIONAL KEY WORDS capillary pressure capillary exchange osmotic and hydrostatic pressure filtration coefficient microcirculation fluid movement across capillary frog mesentery rat omentum filtration-absorption balance

■ Blood flow through the capillary bed depends upon a pressure differential between the arterial and venous ends of the vessels. Quantitative analyses of the factors which influence flow through the microvascular bed would be possible if the precise pressures in the different parts of the system were known. Ideally, such studies should provide repeated pressure recordings in the same vessel or in different parts of the microcirculation without injury to the vessels involved.

In the past, estimates of pressure in the capillary vessels were obtained by several different approaches: the pressure required to compress small vessels to the point where flow ceases, apparently inaccurate and beset with difficulties in interpretation; null methods requiring puncture of capillary vessels with a micropipette, as first used by Landis and Wind and more recently improved by Wiederhielm and Rushmer, who added an electronic microtransducer; direct measurements through microcannulae inserted in the vessels, as used by Rappaport, Bloch and Irwin, successful only down to vessels 30 μ in diameter.

Direct measurements of capillary pressure, requiring micropuncture of the vessel, are subject to the limitation that pressure gauges are not available with sufficiently small volumetric displacements to measure pressure at the end of a micropipette with a tip of 2 to 3 μ. Null point methods circumvent this difficulty by balancing a comparatively large outside source.
of pressure with the pressure of fluid in the capillary, so that measurements can then be made with conventional equipment. All of the direct methods have the drawback of being technically difficult and unpredictable, and of traumatizing the capillary vessel.

There is need for a method which does not require direct puncture of the capillaries. Actually such a possibility exists through the Landis' micro-occlusion technique. The present paper is concerned with the refinement of this technique to increase its reliability and to make it measure fluid movement more precisely.

In the original study of Landis, large numbers of observations were averaged because of the variability encountered; little reliability was placed on single observations. The considerable scatter of the measurements was interpreted as an inherent biological variability. It is, however, equally possible that uncertainties in the method of measurement may have contributed to this variability.

Wind in his studies of pressures by micro-puncture and of fluid movement from capillaries encountered differences due to handling of the preparation, length of exposure, etc. For this reason the exposure techniques utilized in our studies are those that we have developed over a number of years and have standardized.

The current findings indicate clearly that repeated measurements can be made on single capillaries to provide consistent values of fluid movement. By using values of either pressure or filtration coefficients currently in the literature it was found possible to calculate one or the other parameter. In support of the suggested approach, it should be noted that none of the pressures calculated by assuming a filtration coefficient fell outside of the range of values found by investigators utilizing micropuncture methods.

Methods

INDIRECT METHOD FOR MEASURING PRESSURE IN CAPILLARIES

A method of measuring filtration or absorption was devised by Landis who occluded single capillaries in the frog mesentery by compressing them with a microneedle until the flow of blood was stopped. Any subsequent, sustained motion of the red cells trapped in the capillary must be due to a gain or loss of fluid to the surroundings, the assumption being made that a red cell will move with the same velocity as the fluid that it displaces. With the stoppage of blood flow by mechanical occlusion, the pressure on the arterial side of the occlusion comes into equilibrium with the pressure of the nearest supply vessel with an active blood flow. Similarly, on the venous side of the occlusion, the pressure reflects that of the venule into which the capillary empties. These contingencies are shown diagrammatically in figure 1.

According to the Starling hypothesis, fluids exchange between a capillary and the surrounding tissue, driven by the difference in hydrostatic pressure between the plasma Pp and tissue Pt, and the differences in colloidal osmotic pressure of plasma P outward and tissue Pip inward, according to the relation:

\[ \text{Fluid movement} = K \left( P_p - P_t - (\pi_p - \pi_t) \right) \] (1)

In the past, the hydraulic and colloid osmotic pressures in the tissue have been neglected in such calculations, the assumption being that the actual tissue pressure was small (some 1 to 2 mm Hg) and that the extracapillary protein was likewise negligible. In fact, the two values were believed to cancel each other. There is, however, no generally acceptable method of measuring the precise "tissue pressure". Guyton has estimated a negative pressure of some -6 to -7 mm Hg in loose connective tissue by a method using a subcutaneous perforated capsule. Kjellner used an indirect method based on the effect of elevated venous pressure on capillary filtration and

![Figure 1](http://circres.ahajournals.org/)

\[ \text{FIGURE 1} \]

Diagram of the microneedle occlusion technique for estimating capillary pressure. The capillary bed is shown in simplified form and blood or hydraulic pressure and colloid osmotic pressure indicated. Following occlusion, the pressure equilibrates with that of the nearest arterial or venous vessel on either side of the obstruction.
estimated a positive "tissue pressure" of some +5 to +7 mm Hg. Clearly, since both methods are open to criticism, the question of the magnitude of "tissue pressure" in different regions remains controversial. In fact, it is probable that "tissue pressure" does not remain constant but varies considerably. Likewise, there is no clear indication of the precise amount of plasma protein that is present in the extracapillary compartments. Some leakage of protein presumably occurs continuously through the small venules and it has been calculated that for the capillary bed as a whole, the average interstitial colloid osmotic pressure may be from 4 to 5 mm Hg.

In acute experiments, the combined effect of \( P_t \) and \( P_t \) on fluid movement can be assumed to be a constant to which an arbitrary value can be assigned, or which can be neglected at this stage of our work. In the present study, the latter course was adopted because we felt that no meaningful value could be assigned to these tissue factors at present. With this simplification, equation 1 reduces to:

\[
F = K (P_t - P_t) \quad (2)
\]

It can be seen from the above relationship that the capillary pressure can be determined in experiments where the filtration rate \( F \) is measured, providing the colloidal osmotic pressure and the filtration coefficient are known.

The colloidal osmotic pressure of blood can be measured extracorporeally with a membrane osmometer. Filtration coefficients have been established for a limited number of situations and can be used in such determinations. In the original experiments of Landis, the movement of one red cell at a time was followed in order to determine fluid movement. On the basis of this end point alone, it is not possible to decide for any given determination whether the motion is complicated by leakage at the site of obstruction because of injury or incomplete occlusion.

We found that this uncertainty could be avoided by using sequential photographs to track the relative motion of two or more red cells to determine the amount of fluid that is exchanged through the particular capillary wall (fig. 2). In our experience, the occurrence of either filtration or absorption can be deduced only when the whole assembly of trapped corpuscles is seen respectively either to contract or to expand, i.e., the column becomes more dense as water leaves, or more dispersed in the opposite case. The filtration rate at the time of occlusion then can be determined by extrapolating the measurements back to the time at which the flow was mechanically stopped.

Figure 3 illustrates the five types of effects that can take place after occlusion and can be clearly identified when the position of two or more red cells is plotted as a function of time, and the initial slope of their trajectory is determined. The possibilities are:

(a) Movement towards needle on both sides of obstruction at different rates (filtration).
(b) Movement away from needle on both sides of obstruction at different rates (fluid absorption).
(c) Movement toward obstruction on arterial side and away from microneedle on venous side (a corollary of the Starling hypothesis).
(d) Movement at different rates toward needle on both sides of obstruction. However, on each side the individual cells move at uniform rates characteristic for either arterial or venous conditions (injury to wall).
(e) Movement at same rate and in the same direction on both sides of obstruction (incomplete occlusion).

In instances where filtration occurs in occluded vessels, the red cells closest to the obstruction move more slowly than those further away, since the movement of the latter involves a greater loss of fluid per unit time. In the case of injury or incomplete occlusion the different red cells move at the same velocity since the outflow is localized, and the rate is usually greater than that characteristic of normal filtration. Even here, when the motion is not too rapid, a difference in slope can be measured, and this difference reflects the loss of fluid through the wall.

Once the motion of the red cells has been determined, the diameter and length of the capil-
lary through which they have moved establish the filtration rate, and the pressure in the capillary can be calculated from equation 2.

**EXPERIMENTAL PROCEDURE**

The validity of the method as a measure of capillary pressure was tested in the frog mesentery and the rat omentum. Bullfrogs (*Rana catesbeiana*) were either pithed above the cord, or anesthetized with urethane (1 mg/50 g body weight, subcutaneously into the dorsal lymph sac). Rats received...

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**FIGURE 3**

Motion of erythrocytes (rbc) after occlusion of capillary. Any one of five types of movement may be observed after microneedle obstruction of capillary blood flow. The capillary and its respective rbc are shown schematically on the left side of each illustration. The movement of individual red cells towards or away from the needle is plotted in graph form on the right. The actual velocity ($S$ in $\mu$ per second) is estimated by drawing a tangent at the extrapolated zero intercept. (a) represents filtration; (b) absorption; (c) represents the balance between filtration and absorption, as implied by Starling, and is a statistical representation of the capillary bed as a whole with approximately equal filtration and absorption. (d) and (e) are abnormal motions that can be identified when the motions of two or more rbc are followed.
1 mg of urethane/100 g body weight, intramuscularly or 3.0 mg sodium pentobarbital/100 g. No differences were observed between the two forms of anesthesia. The mesentery was exposed through a lateral incision and the animal was placed on a lucite board, under a microscope, with the mesentery supported by a solid block of clear lucite through which it was transilluminated. The preparation was kept moist by a drip of the appropriate Ringer’s solution. To obstruct the flow, a glass microneedle held in a Leitz micromanipulator was used. The tip was shaped into a flat hair-like bend so that the shaft of the final bend could be positioned across the capillary and subsequently brought down firmly until the flow was stopped. At the instant the flow was stopped, a signal was recorded on a chart recorder run at a paper speed of 10 mm/sec. To determine the subsequent position of red cells, 35 mm photomicrographs of the field were taken at 5- to 10-second intervals with a 1/500 second exposure. The exact moment of exposure was marked electrically on an adjacent channel of the recorder.

A given sequence of pictures was enlarged on 8" x 12" paper, and from these prints the position of red cells was measured as a function of time by selecting a clearly identifiable reference point common to the sequence. Since the magnification was not the same in all experiments, the actual dimensions were determined by measuring the average size of the red cells and converting this to microns according to measured values. In most experiments a special long working distance objective (Leitz UM 32) with a magnification of 22x was used in connection with a 10x ocular.

**Results and Discussion**

Results obtained in the frog mesentery are shown in table 1. Pressures were calculated by assuming a colloidal osmotic pressure of 7.6 mm Hg and a filtration coefficient of \( \frac{0.00816 \, \mu^3}{\mu^2 \cdot \text{sec} \cdot \text{mm Hg}} \) as reported by Landis. It can be seen that the pressures calculated from the present measurements fall into the upper range of values reported by Landis. Evidence of water absorption from the tissue into the capillary was found in less than 15% of the experiments made.

Results for the rat omentum are shown in table 2. Pressures were calculated by assuming a colloidal osmotic pressure of 19.5 mm Hg as reported by Keys and Hill and a capillary filtration coefficient of 0.01 \( \frac{\mu^2}{\mu^2 \cdot \text{sec} \cdot \text{mm Hg}} \). This coefficient was arrived at by assuming that the maximum filtration rate would correspond to the maximum pressure in the capillary of this preparation, which has been reported by Landis to be of the order of 25 mm Hg. As in the case of frogs, evidence of absorption of fluid from the tissue into the capillary was found for less than 15% of the experiments made, irrespective of

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**TABLE 1**

**Determinations of Capillary Pressure in Frog Mesentery**

| Exp. no. | Type of vessel | Site of occlusion | Velocity difference between red cells | Vessel diameter | Distance between cells | Filtration rate per unit area | Calculated pressure
<table>
<thead>
<tr>
<th></th>
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<tr>
<td>3</td>
<td>Arteriolar</td>
<td>A†</td>
<td>1.54</td>
<td>17.0</td>
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<td>capillary</td>
<td>V</td>
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<td>23.0</td>
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<td>0.015</td>
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<tr>
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<td>V</td>
<td>1.34</td>
<td>20.5</td>
<td>72</td>
<td>0.063</td>
<td>18.9</td>
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<td>9</td>
<td>Midcapillary</td>
<td>A</td>
<td>1.31</td>
<td>16.3</td>
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<td>0.049</td>
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<td>V</td>
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<td>20.0</td>
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<td>0.034</td>
<td>11.7</td>
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<td>3.69</td>
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<td>11.6</td>
<td>60</td>
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<td>10.4</td>
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<td>0.17</td>
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<td>V</td>
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<td>25.2</td>
<td>45</td>
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<td>(-)§</td>
<td>3.0</td>
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*Values are representative of 66 occlusion experiments in 15 frogs (Rana catesbeiana).†Pressures at both sides of the occlusion are calculated by assuming a colloidal osmotic pressure of 7.6 mm Hg and a filtration coefficient of \( \frac{0.00816 \, \mu^3}{\mu^2 \cdot \text{sec} \cdot \text{mm Hg}} \).‡A: on arterial side of occluding microneedle. V: on venous side.§(—): absorption.

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**Table 2**

Determinations of Capillary Pressure in Rat Omentum*

<table>
<thead>
<tr>
<th>Exp. no.</th>
<th>Type of vessel</th>
<th>Site of occlusion</th>
<th>Velocity difference between red cells (μ/sec)</th>
<th>Vessel diameter (μm)</th>
<th>Distance between cells (μm)</th>
<th>Filtration rate per unit area (μl/μl/sec/mm Hg)</th>
<th>Calculated pressure (mm Hg)</th>
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<tbody>
<tr>
<td>29</td>
<td>Capillary</td>
<td>A±</td>
<td>1.24</td>
<td>14.5</td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>venule</td>
<td>venule</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>29</td>
<td>Precapillary,</td>
<td>A</td>
<td>1.38</td>
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<td>72</td>
<td>0.075</td>
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<tr>
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<td>A</td>
<td>0.51</td>
<td>17.0</td>
<td>36</td>
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<tr>
<td>72</td>
<td>Capillary,</td>
<td>A</td>
<td>0.19</td>
<td>8.5</td>
<td>36</td>
<td>0.012</td>
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<tr>
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<td></td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

*Values are representative of findings in 27 occlusion experiments on 9 rats, of 200 g average weight.

†Pressures at both sides of the occlusion are calculated by assuming a colloidal osmotic pressure of 19.6 mm Hg and a filtration coefficient of 0.01 μl/μl/sec/mm Hg.

‡A: on arterial side of occluding microneedle. V: on venous side.

The anesthetic used. It should be pointed out that measurements made on collecting venules (18 to 25 μ wide) showed outward filtration despite the fact that the hydraulic pressure in these vessels should be below that in the capillaries proper.

The present method based on an analysis of the relative motions of two or more trapped red cells avoids most of the uncertainties of the events taking place after occlusion because leakage due to injury or incomplete occlusion can be readily identified.

The accuracy of the blood pressures which we have calculated by this method will depend upon the accuracy of the method by which the filtration coefficient for capillary vessels was determined originally. In the present calculations we assumed that the different components of the capillary bed have a uniform coefficient of permeability and used the value obtained by Landis on the basis of an average of all of his measurements. Inasmuch as none of the calculated pressures was higher than those in previous reports, it seems plausible to assume that the filtration coefficient does not vary significantly within this general range in different parts of a particular capillary bed.

A possible source of error in such measurements stems from the use of the motion of the red cells as an indicator of fluid movement. The majority of the observations were made on narrow capillaries holding only a column of single red cells. Single cells appeared to move freely after the vessel had been obstructed by the microneedle and even aggregates of two or three red cells had no difficulty in moving towards or away from the microneedle.

Since this method measures pressures at the no-flow condition, the actual values during established flow should be slightly lower to account for the entrance losses. The method permits simultaneous measurement of pressure conditions at entrance and exit, and it should be applicable to rheological measurements in vivo. For example, effective blood viscosity in capillaries could be determined in such experiments by recording the velocity of flow just prior to the occlusion.

Inspection of equation 2 shows that the micrurgical method, as described above, can be used in an alternative way to determine...
simultaneously the hydraulic pressure and the filtration coefficient. The colloidal osmotic pressure can be altered by the intravenous infusion of a given amount of albumin. Under these conditions, by repeating the occlusive test procedure before and after the infusion and measuring the colloidal osmotic pressure for both conditions, two sets of equations can be derived from equation 2. If one assumes in an acute experiment that at a given location in a capillary the hydrostatic pressure and the filtration coefficients remain the same in both states, these parameters can then be solved by:

$$P_s = \frac{F_1 \pi_{p2} - F_2 \pi_{p1}}{F_1 - F_2} \quad (3)$$

$$K = \frac{F_1 - F_2}{\pi_{p1} - \pi_{p2}} \quad (4)$$

where subscripts 1 and 2 denote the two conditions.

References
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